Anti-Tubulin, Beta-III
Immunohistofluorescence Protocol

Catalog #: 2020-TUB
Species: mouse
Tissue: Mouse hippocampus

Fixation: 4% paraformaldehyde 18 hours
Antibody incubation: Primary Antibody: 4C, overnight and RT, 1 hour Secondary Antibody: RT, 1 hour
Antigen Retrieval: 10mM citrate buffer (pH 6.0, 0.05% Tween 20)

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde in PBS
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **PBST:** 0.4% Triton-X in 1X PBS
- ✓ **Blocking buffer:** 10% goat serum in PBST
- ✓ **Incubation buffer:** 2% goat serum in PBST
- ✓ **Secondary Antibody:** examples used is a Goat-Anti-Rabbit Alexa Fluor 488, ThermoFisher (catalog # A-11034) and Goat-Anti-Mouse Alexa Fluor 647, ThermoFisher (catalog # A-21236)
- ✓ **Mounting media:** Permount, Fisher Scientific (catalog # SP15-100)
- ✓ **Counterstain:** DAPI, ThermoFisher (catalog # D1306)

Before you begin

This protocol was used for tissues fixed without perfusion following standard FFPE protocol. Cut tissue into 3-5mm sections and place in 4% paraformaldehyde in PBS overnight at 4C. For proper fixation, submerge sections into a 20x volume of fixative based on the mass of the tissue. After fixation of tissue, dehydrate and embed tissue into paraffin blocks according to standard protocol. Then section the blocks at 8 microns. Finally, transfer the sections onto positively charged slides (example: SFH1103, BioCare Medical) and dry overnight at room temperature.

Deparaffinize

1. Warm slides for 10 minutes in a 60C oven.
2. Incubate slides in the following dehydrants in this order
   - I. Xylene: 3 times, 10 minute intervals
   - II. 100% ethanol: 2 times, 5 minute intervals
   - III. 95% ethanol: 2 times, 3 minute intervals
   - IV. 80% ethanol: 2 times, 1 minute interval
   - V. H2O: 2 times, dip to rinse.

Antigen Retrieval

1. Place slides in 10mM citrate buffer (pH 6.0, room temperature) for 30 minutes.
2. Wash slides with 1X PBS.

Immunohistochemistry

1. Block slides with blocking buffer for 30 minutes at RT.
2. Wash slides with PBST 3 times, in 15 minute intervals.
3. Dilute Anti-CNPase (Cat. # 325-CNP) to 1:500 in incubation buffer. Incubate sections overnight at 4C.

4. Wash slides with PBST 3 times, in 15 minute intervals.
5. Dilute Anti-βIII Tubulin (Cat. #2020-TUB) to 1:1000 in incubation buffer. Incubate sections for 1 hour at room temperature.
6. Wash slides with PBST 3 times, in 15 minute intervals.
7. Dilute secondary antibodies in incubation buffer per manufacturer's recommendation. Incubate sections for 1 hour at room temperature with each secondary individually. Wash slides with PBST 3 times, in 5 minute intervals between secondary antibodies.

   Tech Tip:

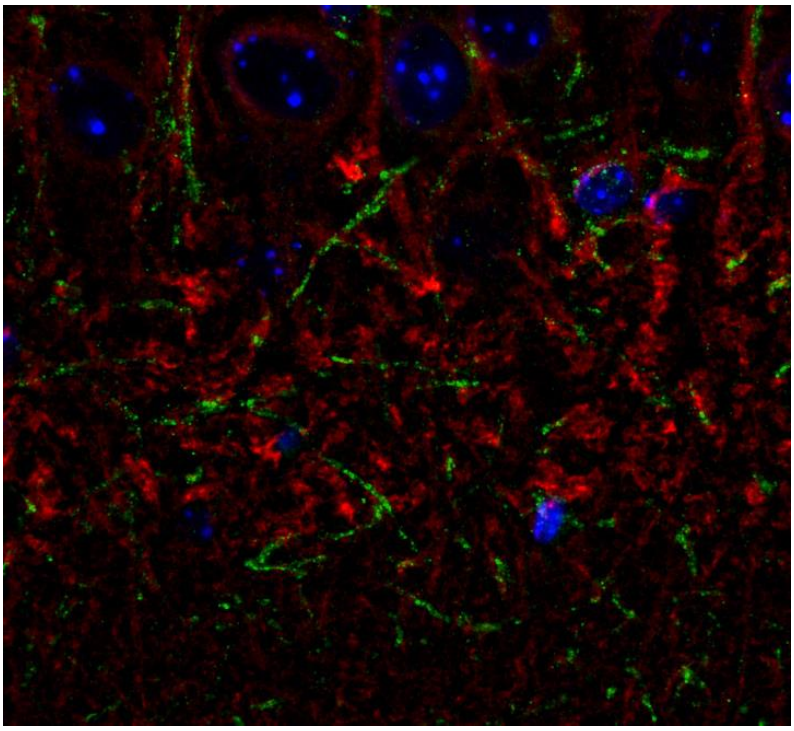
   a. A goat anti-mouse Alexa Fluor 647 antibody was used to visualize the βIII tubulin in the image below, ThermoFisher (catalog # A-21236, 1:1000). Any anti-rabbit secondary can be used.
   b. A goat anti-rabbit Alexa Fluor 488 antibody was used to visualize the CNP in the image below, ThermoFisher (catalog # A-11034, 1:1000). Any anti-rabbit secondary can be used.
8. Remove secondary antibody and wash slides with PBST 3 times, in 15 minute intervals.
9. Prepare fresh DAPI solution per manufacturer's recommendation. Apply to slide and rinse 5 times with PBS.

   Tech Tip:

   a. Any nuclear counterstain can be used, for this protocol DAPI was used, catalog # D1306.
10. Apply mounting medium onto slide and gently place glass cover slip before viewing under the microscope.

    Tech Tip:

    a. Any mounting media can be used, for this protocol Permount was used, catalog # SP15-100.



Immunolabelling of the CA3 subfield of mouse hippocampus labeling CNP (Cat# 325-CNP, green, 1:500) and β-III tubulin(Cat# 2020-TUB, 1:1000, red). The blue is DAPI staining DNA. Original magnification is 40X. Antibody sections were incubated in anti-CNP overnight at 4°C and in anti-βIII tubulin for 1hr at room temp. Photo courtesy of Rob Wine.